ACTIVATION MECHANISM OF HUMAN COLLAGEN RECEPTOR INTEGRINS: STRUCTURE AND FUNCTION OF INTEGRIN α1ß1 AND α2ß1 I-DOMAINS

Liisa Pösö[1], Doc. Jarmo Käpylä[1], Doc. Tomi Airenne[2], Prof. Tiina Salminen[2] and Prof. Jyrki Heino[1]
[1]Department of Life Technologies, University of Turku, [2]Faculty of Science and Engineering, Åbo Akademi University

BIOCHEMISTRY

INTRODUCTION

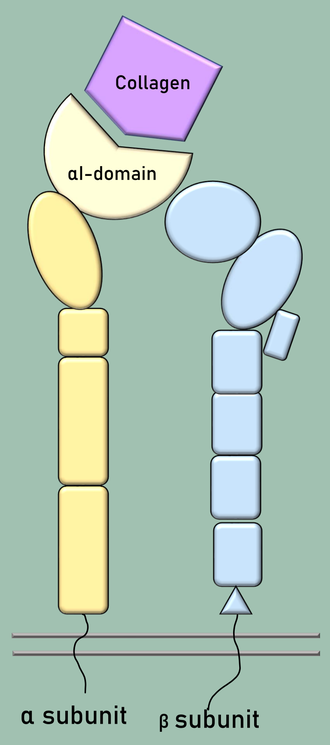


Figure 1.
General integrin structure

Extracellular matrix integrins α1β1 and α2β1 are collagen receptor integrins that participate in multiple cellular functions and play a crucial role in cell-cell interactions. Recognition of collagen occurs via the α subunits I-domain (Figure 1.), which activates the complete integrin macromolecule amid collagen binding. Collagen binding is made possible through conformational change in the I-domain which is consequential of an ion bond between the domains αC and α7 helix breaking. The bond breaking allows the two α helixes to move away from each other, creating a more open conformation for the I-domain and thus allowing a collagen molecule to bind to its specific binding site. α1I-domain is known to have a transitional conformation that occurs between the two main conformations which was discovered through site-directed mutagenesis of the α7 helix glutamic acid. However, a similar conformation for the α2I-domain has yet to be discovered.

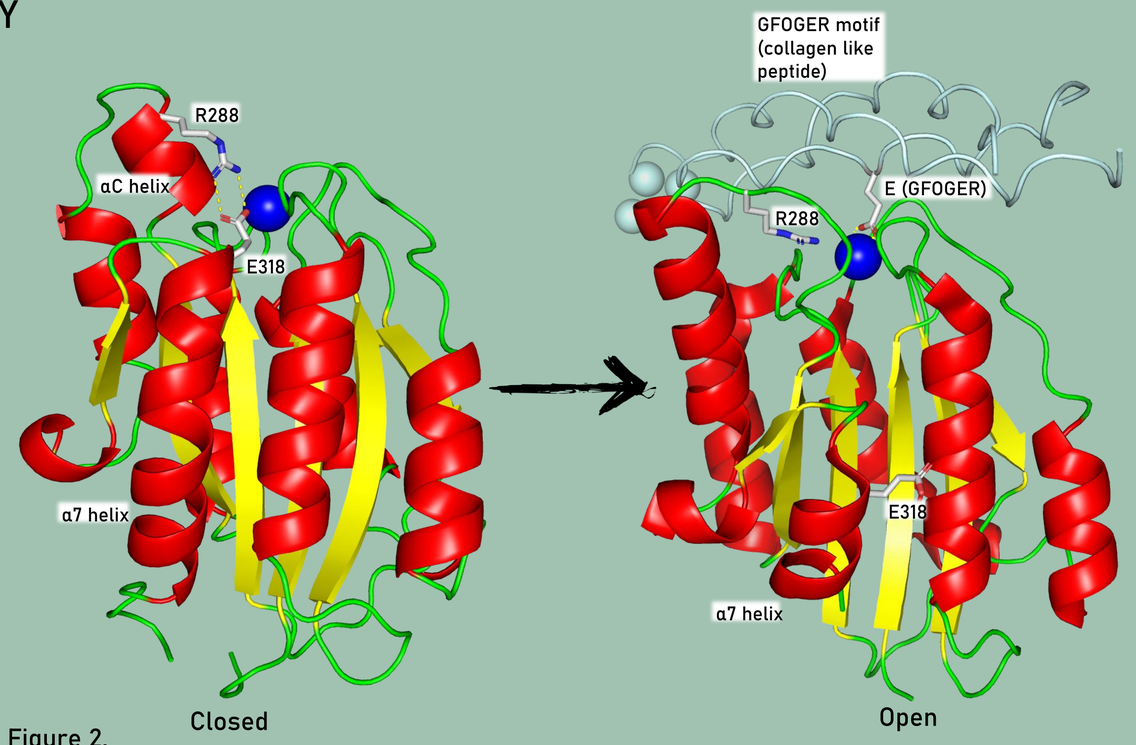


Figure 2.
Conformational change of α2I-domain from closed structure (PDB ID: 1AOX) to open, collagen binding structure (PDB ID: 1DZI). Important amino-acids and mobile helixes have been labelled. Catalytic metal ion is shown as a blue sphere and ion bonds are represented with a yellow dotted line.

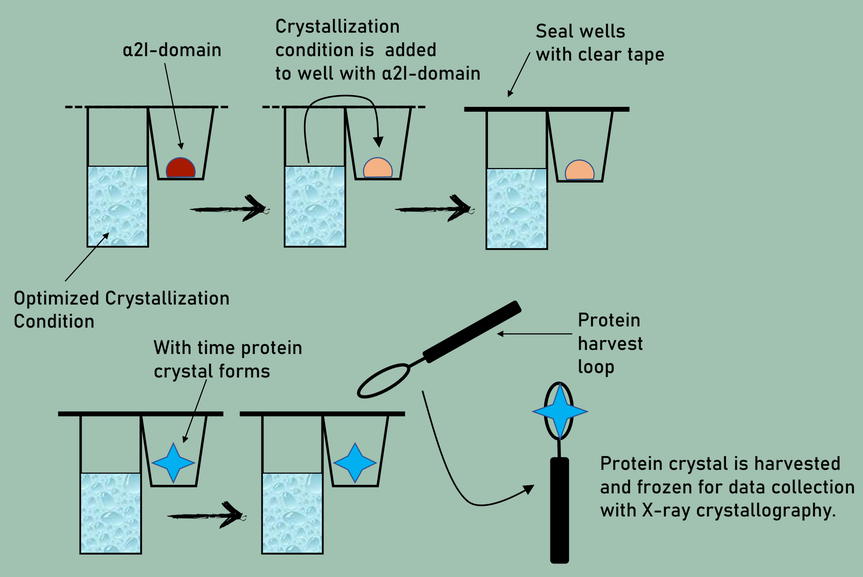
AIMS

1. To determine the αC helical arginine's role in the activation mechanism of both integrins and whether they have a mechanistical difference.
2. To establish if there is a novel conformation to be found in the activation mechanism of α2β1 I-domain.

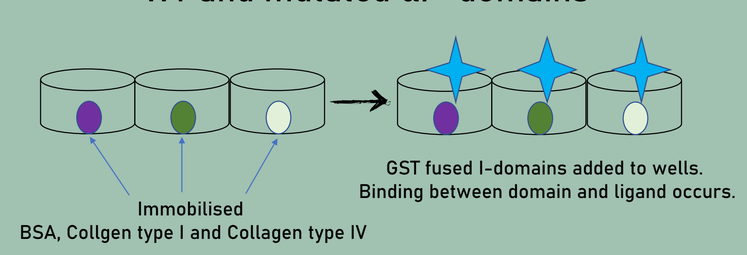
MATERIALS AND METHODS

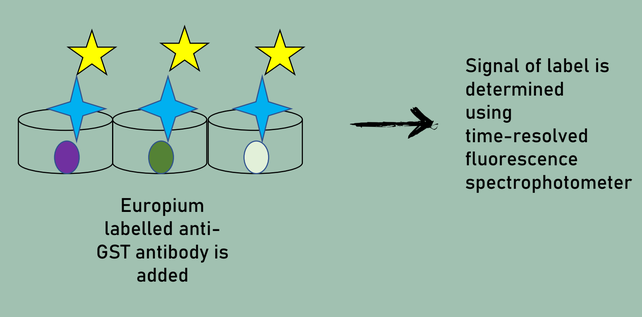
GST fused α1I- and α2I-domains were expressed in *E. coli* and extracted with affinity chromatography using a Glutathione-Sepharose column.

1. Sitting Drop Vapor Diffusion Crystallisation of α2I-domain carrying R288A mutation



2. Solid Phase Binding Assay with WT and mutated αI –domains





RESULTS AND CONCLUSION

According to binding assays (Fig.3) performed with both integrin I-domains and their mutations, it can be concluded that there is a clear mechanistical difference in the activation mechanisms of α1I- and α2I-domains. Arg-288 seems to have a larger role in the activation of the α2I-domain. The corresponding arginine in α1I-domain does not seem to have a significant role in its activation mechanism. When comparing all mutants to the wild-type I-domains it can be concluded that both mutants are gain of function mutations.

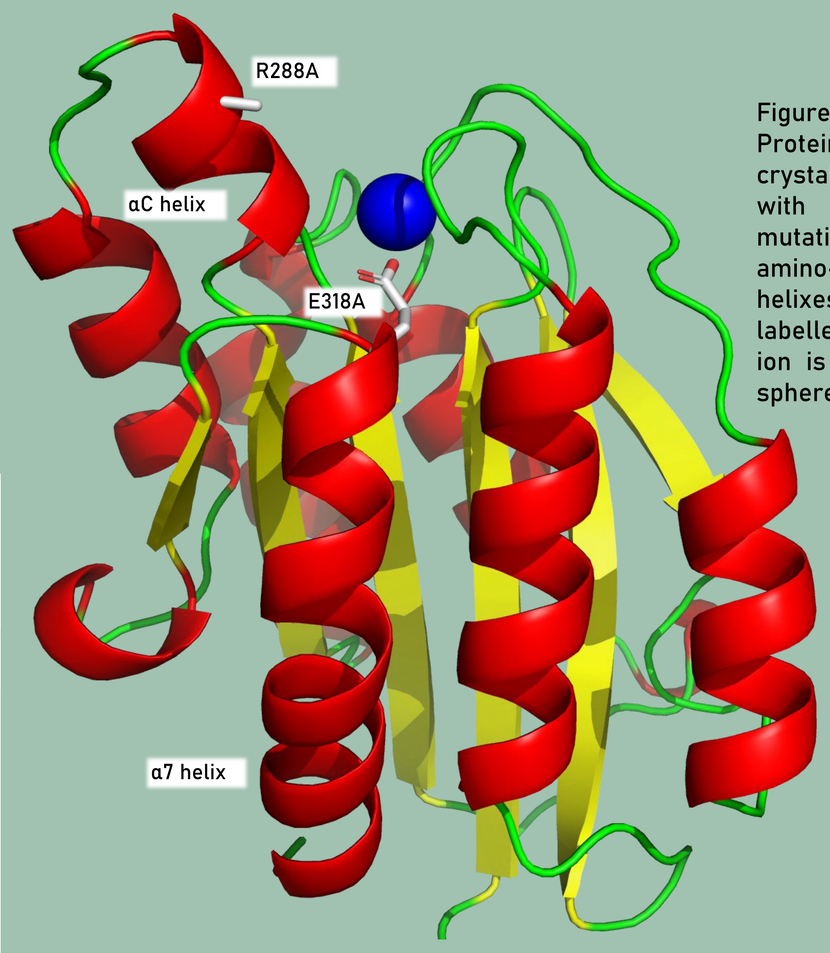


Figure 4.
Protein structure of crystallized α2I-domain with R288A point mutation. Important amino-acids and mobile helixes have been labelled. Catalytic metal ion is shown as a blue sphere.

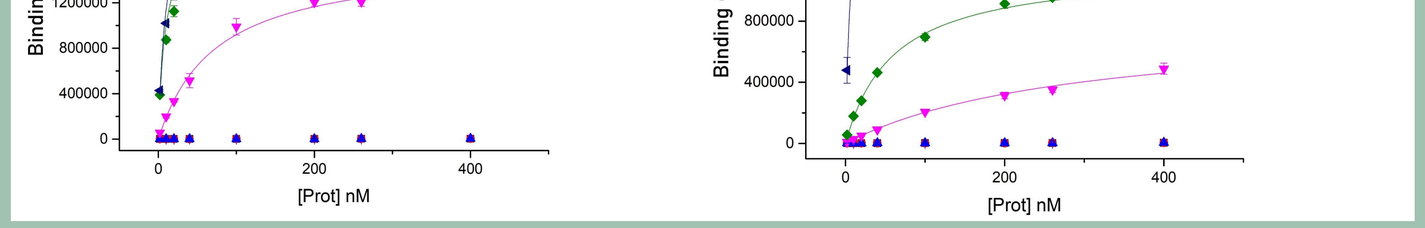


Figure 3.
Binding of α1 and α2 I-domains to collagen type I and collagen type IV. BSA is used as negative control. Data was fitted to equation binding=Bmax/(1+Kd/[aldomain]), where Bmax is maximal binding and Kd is apparent value for dissociation constant.

Crystallization optimisation allowed for the α2I-domain with R288A point mutation to be successfully crystallised. Data from the gained diffraction map was processed with appropriate software and a 3D structure was formed (Figure 4.). Initial analysis of the structure did not reveal a significant difference to the closed conformation of the wild-type (Fig.2).